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A New Model Compound for Studying Alkaline Cellulose Chain Cleavage Reactions

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A NEW MODEL COMPOUND FOR STUDYING ALKALINE CELLULOSE CHAIN CLEAVAGE REACTIONS

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ABSTRACT

A conformationally rigid cellulose model, the 4,6-O-benzylidene of 1,5-anhydrocellobiitol, has been studied to learn more about the mechanisms of chain cleavage reactions under alkaline pulping conditions. Heating the model at 170°C in 2.5N NaOH gave 55% glycon-oxygen (G-O) bond cleavage, and ~45% oxygen-aglycon (O-A) bond cleavage.

The amount of observed O-A bond cleavage is significantly higher than that for 1,5-anhydrocellobiitol, which is a more conformationally flexible cellulose model. The benzylidene model also degraded about ~35% faster than 1,5-anhydrocellobiitol; much of this rate increase can be attributed to a faster rate of O-A bond cleavage for the benzylidene. These results point out the value of choosing appropriate cellulose models.

INTRODUCTION

The stringent conditions (170°C, 2.5 N NaOH) of chemical pulping lead to as much as a 10% loss in cellulose yield,[1] together with a factor of three or more decrease in degree of polymerization of the cellulose.[1-3] The latter results in lower pulp viscosity and, hence, pulp properties. The yield loss is a result of the stepwise removal of individual glucose units from the reducing end of the cellulose polymer chain (i.e., peeling); the molecular weight is only slightly affected. In contrast, drastic reductions in the molecular weight are attributed to the random cleavage of the glycosidic bonds linking the glucose units of cellulose together.[4]

The study reported here focuses on learning more about the mechanisms responsible for cellulose chain cleavage in highly alkaline solutions. Many previous studies have also addressed this area; most have involved model compounds rather than cellulose itself. Early models employed phenyl glycosides (**1**) and alkyl glycosides (**2**). The major proposed mechanism for glycosidic bond cleavage for these models, as well as cellulose, is an $S_N$icB(2) mechanism (Figure 1).[5,6] The $S_N$icB(2) refers to a nucleophilic substitution by an internal nucleophile - the conjugate base of the C2 hydroxyl group.

The $S_N$icB(2) mechanism requires that the molecule have a conformation such that the C1 and C2 substituents are trans diaxial to each other. The trans diaxial conformation permits backside attack by the C2 oxyanion at C1, displacing the aglycon. The carbohydrate molecule would have to flip from its more conformationally stable all-equatorial form to its all-axial conformation in order to have a trans diaxial conformation. Such a flip would be impossible for crystalline cellulose and probably difficult for amorphous cellulose polymer segments.

More recent studies have involved disaccharides (**3**) as the model compounds.[7-9] These compounds, which are more structurally similar to cellulose, have a nonreducing sugar as the aglycon. The studies found that a number of mechanisms were responsible for the alkaline degradation of glycosidic bonds; these included an S_N1 mech-

anism, an $S_N$icB(2), and another neighboring group mechanism, $S_N$icB(2)-ro. The latter involves ring opening of the glycon as an intermediate step (see Figure 1).

In this paper, we report the results of an evaluation of the alkaline degradation of a new model compound having a more restricted conformational mobility than previously studied models. This restricted mobility would make the model compound more similar to the rigid cellulose polymer chains found in wood and pulp. The new model is 1,5-anhydro-4-O-(4,6-O-benzylidene-β-D-glucopyranosyl)-D-glucitol (**4**). This nonreducing disaccharide, shown in Figure 2, will be referred to as benzylidene 1,5-anhydrocellobiitol.

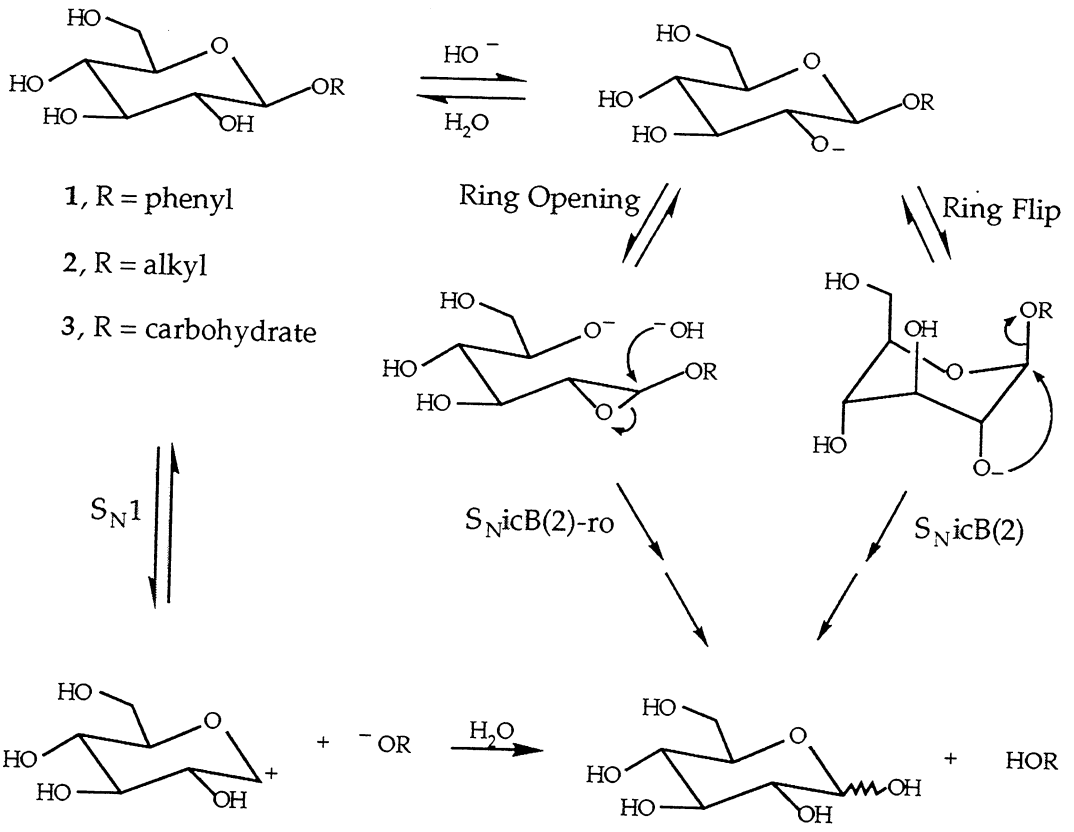


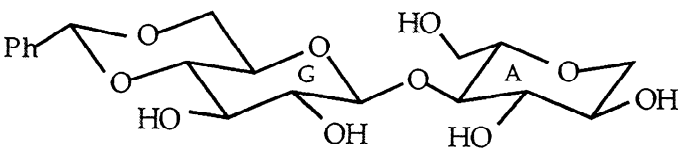
Figure 1. Mechanisms for Glycosyl-Oxygen (G-O) Bond Cleavage.

RESULTS

Design/Preparation of the Model Compound

The model compound (**4**) selected for this research is similar to cellulose in that it has both glycosyl-oxygen (G-O) and oxygen-aglycon (O-A) linkages; it should also possess limited molecular flexibility because of a 4,6-O-benzylidene linkage. The benzylidene linkage prevents the molecule from flipping to an all axial chair conformation, the first step proposed for the $S_N$icB(2) mechanism. An $S_N$icB(2) mechanism may still be feasible, however, since the requisite 1,2-trans diaxial conformation is possible if the benzylidene 1,5-anhydrocellobiitol converts to its boat conformation.[10] This conversion would have some energy constraints, since the boat form is reported to be 10 kcal/mol less stable than a chair conformation for glucopyranose rings. This value should be viewed as a minimum for cellulose, due to constraints of bond rotation and hydrogen bonding.[11]

The disaccharide benzylidene model (**4**) was prepared by reacting 1,5-anhydrocellobiitol (structure **8** in Figure 2) with α,α-dimethoxytoluene in the presence of a catalytic amount of *p*-toluenesulfonic acid and was purified by recrystallization. Likewise, a monosaccharide model, 4,6-O-benzylidene-1,5-anhydro-D-glucitol (**5**), was prepared from 1,5-anhydro-D-glucitol. This simpler model was needed to verify the stability of the benzylidene linkage. This linkage is known to be base-stable under ordinary conditions;[12] however, our conditions are not ordinary. The nonreducing sugar portion of this model, 1,5-anhydro-D-glucitol, is known to be stable under pulping conditions.[7]



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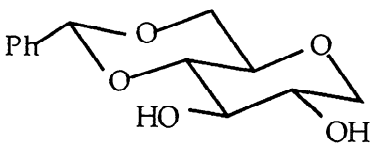

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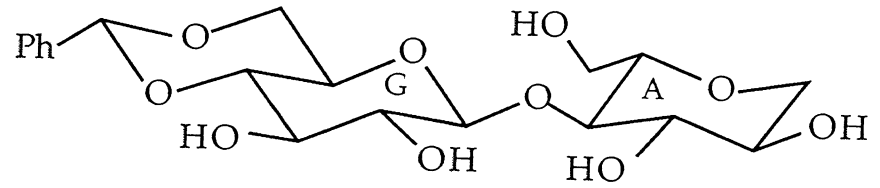
Compound **5** was heated at 170°C in 2.5N NaOH. The rate of disappearance of **5**, using a pseudo-first-order kinetics analysis,[9] was found to be 40 times slower than the disappearance rate of compound **4** under similar conditions. This demonstrated that the benzylidene linkage cleaves much more slowly than the glycosidic linkage in **4**.

Identification of the Alkaline Degradation Products

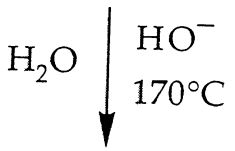
Alkaline degradation of benzylidene 1,5-anhydrocellobiitol gave both alkali-stable and alkali-labile products (Figure 2). The alkali stable products, principally formed from the non-reducing (aglycon) portion of the compound, were identified by comparing gas chromatographic (GC) retention times and GC/mass spectra to known compounds. The expected aglycon product, 1,5-anhydro-D-glucitol (**6**), was found in 55% ± 2% yield. A second aglycon product, 1,5:3,6-dianhydro-D-galactitol (**7**), was produced in 32% ± 5% yield. In addition, 0.5-2% of 1,5-anhydrocellobiitol (**8**) was formed, presumably by the degradation of the 4,6-O-benzylidene linkage. Compound **8** will break down to form additional 1,5-anhydro-D-glucitol;[7] however, as we will show, the rate is slower than degradation of **4**. The 1,5-anhydro-D-glucitol coming from **8** represented only a small fraction of the overall source of 1,5-anhydro-D-glucitol and, thus, did not interfere with our study.

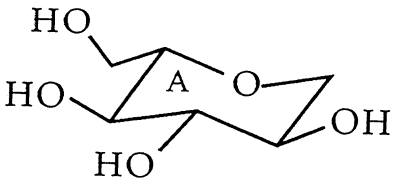
Some products could not be identified by GC/MS and were deduced to be polar, secondary degradation products of alkali-labile 4,6-O-benzylidene-D-glucopyranose (**9**). The latter, which contains a reducing end group, would be formed from either G-O or A-O bond cleavage and would quickly degrade by peeling-type reactions to smaller fragments under the reaction conditions. A sample of **9** was prepared and degraded under the same conditions (2.5 N NaOH, 170°C) to confirm this hypothesis. After heating for 5 hours, the solution containing **9** was worked up in the same way as **4**. The GC analysis showed that 26 signals from the degradation of the glucose benzylidene **9** had identical retention times to the "unidentified" signals found in the alkaline degradation of the model compound **4**.



4

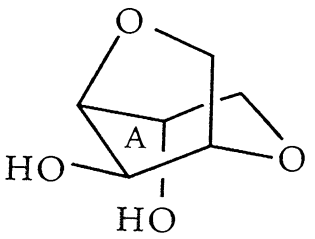
G = Glycon side
A = Aglycon side





6 (55%)

+



7 (32%)

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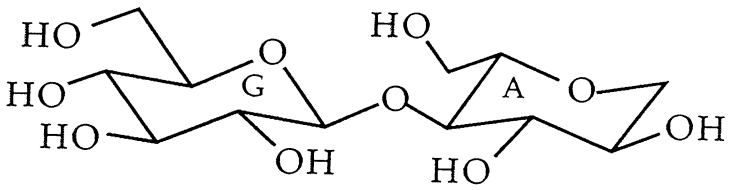

8 (~1%)

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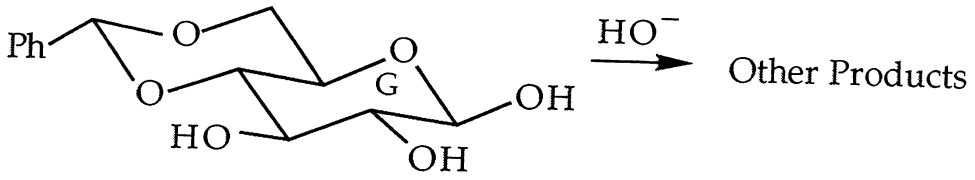

9

Figure 2. Alkaline Degradation Products from **4**.

A 170°C degradation of the model compound **4** in the presence of ^{18}O-enriched aqueous sodium hydroxide (2.5 N NaOH) led to 1,5-anhydro-D-glucitol having no ^{18}O enrichment. This indicated that all of the 1,5-anhydro-D-glucitol was formed by G-O bond cleavage.

Kinetic Analysis of the Degradation Reaction

The disaccharide model **4** (0.01 M) was allowed to react in aqueous sodium hydroxide (0.6 or 2.5 N) at 170°C. Samples were taken from 2 to 98 hours; the rate of disappearance of the model compound, and the rate of appearance of its degradation products were then followed. Pseudo-first-order kinetics were assumed since a 250-fold excess of hydroxide ion was used at constant ionic strength. Values for k_r, the pseudo-first-order rate constant for the degradation of the model compound, were determined using Eq. (1):

$$\ln R = -k_r t + \ln R_o \qquad (1)$$

where R is the concentration of the reactant at time t, and R_o is the initial reactant concentration.

Kinetic analysis of product formation was also done assuming pseudo-first-order kinetics. The calculations have been described previously by Brandon et al.,[7] and include the following equations:

$$(P_{i,t} - P_{i,o}) = k_i(R_o/k_r)\,[1-\exp(-k_r t)] \qquad (2)$$

$$X_{i,\infty} = P_{i,f}/(R_o - R_f) \qquad (3)$$

$$k_i = k_r * X_{i,\infty} \qquad (4)$$

where P_i is the product concentration at time t, initially (o), or final time (f); k_i and k_r are the pseudo first-order rate constants for the appearance of product i and disappearance of the reactant, respectively; and $X_{i,\infty}$ is the mole fraction of product i at completion.

Four separate studies were done for the alkaline degradation of benzylidene 1,5-anhydrocellobiitol (**4**) in 2.5 N NaOH, and three studies were done for the alkaline degradation of 1,5-anhydrocellobiitol (**8**). The observed degradation rates ranged from 10.0-11.4 x 10^{-6} sec^{-1} for the benzylidene **4**, and 7.2-8.7 x 10^{-6} sec^{-1} for compound **8**. Previous investigators observed a rate constant of 7.9 x 10^{-6} sec^{-1} for compound **8**, which is exactly in the middle of our range. If we use this value for **8** and our average value for **4** (10.7 x 10^{-6} sec^{-1}), we come to the conclusion that the benzylidene 1,5-anhydrocellobiitol degrades ~35% faster than 1,5-anhydrocellobiitol.

This surprising rate difference was further confirmed by performing an additional degradation experiment in which both compounds were place in the same heating bath and reacted for the same time periods. In this *limited* data set, the benzylidene compound degraded 40% faster: $k_{cpd\ 4}$ = 11.1 x 10^{-6} sec^{-1} and $k_{cpd\ 8}$ = 7.7 x 10^{-6} sec^{-1}.

Effect of Hydroxide Ion Concentration at Constant Ionic Strength

The rate of degradation of **4** was also determined at a lower NaOH concentration. The hydroxide concentration was decreased by 75% (to 0.62 N), while at the same time, the ionic strength of the reaction medium was kept constant (2.5 N) by adding a NaCl solution. The results of this experiment showed a 30% decrease in the degradation rate; namely, the overall rate was 7.0 ($\pm$ 0.1) x 10^{-6} sec^{-1}.

Again, an experiment was conducted where a sample of the benzylidene disaccharide **4** in 2.5 N NaOH was directly compared to a sample of **4** in 0.62 N NaOH/1.87 N NaCl by heating both sets of samples in the sand bath at the same time. The results confirmed that the alkaline degradation of compound **4** was significantly slower (36%) when the concentration of hydroxide was decreased by 75%: $k_{2.5\ N\ NaOH}$ = 11.1 x 10^{-6} sec^{-1}, and $k_{0.6\ N\ NaOH}$ = 7.1 x 10^{-6} sec^{-1}.

The product distribution also changed at the lower NaOH level. The mole fractions from each experiment, calculated using Eq. (4), were averaged to obtain the value for $X_{i,\infty}$ listed in Table 1. The rate con-

stants (k_i values) were calculated for both G-O bond cleavage (obtained by multiplying $X_{Gluc,\infty} * k_r$), and O-A bond cleavage (obtained by multiplying $X_{DAG,\infty} * k_r$); these values are also shown in Table 1.

TABLE 1

Product Mole Fractions and Rate Constants for the 170°C Alkaline Degradation of Benzylidene (**4**) and 1,5-Anhydrocellobiitol (**8**).

	Conc. (N)		Mole Fraction		Rate Constant ($x10^6$)	
Substrate	NaOH	NaCl	$X_{Gluc,\infty}$	$X_{DAG,\infty}$	k_{G-O}	k_{O-A}
4	2.5	0	0.55	0.31	5.6	3.1
4	0.6	1.9	0.47	0.29	3.3	2.0
8[a,b]	2.5	0	0.92	0.10	7.3	0.9

[a]Data from Brandon et. al.[7]

[b]Blythe and Schroeder observed mole fractions of 0.88 and 0.10 for **6** and **7**, respectively, for reacting **8** at 170°C in 1.0N NaOH.[13]

We observed mole fractions of 0.77 and 0.24 for **6** and **7**, respectively, for reacting **8** at 170°C in 2.5N NaOH. Previous investigators[7,13] have found mole fractions of 0.9/0.1 for **6** and **7**, as reported in Table 1. We are uncertain of the reasons for this difference in product distributions. The amount of **7** in our case may have been inflated by a co-eluting GC impurity; we looked for impurities and found none. Since the two earlier investigators found the same mole fractions, we will use their values for discussions that follow. Just as with previous studies[7,13] on the degradation of **8**, we observed small amounts of 1,6-anhydro-β-D-glucopyranose (levoglucosan) as a product. Levoglucosan is an expected product of an $S_N$icB(2) mechanism from a model that has an available C6-OH group; it should not form in the case of benylidene **4** and, indeed, none was observed.

DISCUSSION

Points of Bond Cleavage

The glycosidic linkage in benzylidene 1,5-anhydrocellobiitol can cleave in one of two places: between either the glycosyl-oxygen (G-O) bond or the oxygen-aglycon (O-A) bond. The major stable product, 1,5-anhydro-D-glucitol, was formed in 55% yield and only by G-O bond cleavage (as established by an ^{18}O experiment).

Evidence for cleavage at the O-A bond was the formation of 1,5:3,6-dianhydro-D-galactitol (**7**) in 32% yield. This compound was previously reported as an alkaline degradation product of 1,5-anhydro-cellobiitol,[7] although the yield was only 10% in this case.

Roughly 13 mole % (± 7%) of the aglycon failed to form detectable GC products. These products were most likely formed by oxygen-aglycon (O-A) cleavage since glycosyl-oxygen (G-O) cleavage always produces the detectable, stable 1,5-anhydro-D-glucitol. Thus, product analyses indicated that benzylidene 1,5-anhydrocellobiitol underwent glycosidic bond cleavage at the G-O bond 55% of the time and at the O-A bond ~45% of the time, of which 32% was actually observed.

Mechanisms of Glycosyl-Oxygen (G-O) Bond Cleavage

S_N1 Mechanism

An S_N1 mechanism, shown in Figure 1, involves a heterolytic bond cleavage to produce a carbocation intermediate on the glycosyl side (at the C1) and an anion on the aglycon side. The carbocation is quickly attacked by water or hydroxide ion to form 4,6-O-benzylidene-D-glucopyranose (**9**); this compound is not stable under the reaction conditions, and undergoes peeling reactions to other products (primarily carboxylic acid compounds).[14]

The S_N1 reaction produces alkali-stable 1,5-anhydro-D-glucitol (**6**) from the aglycon side of the molecule. As Figure 1 indicates, if the reaction was done in an ^{18}O-enriched liquor, no ^{18}O would be incorpo-

rated into **6** by an S_N1 mechanism: indeed, this was the observation.

In an S_N1 reaction, the nucleophile does not participate in the rate-determining step; thus, the rate of the reaction should not change with a change in the concentration of the nucleophile.[15] Instead, a 30% decrease in the reaction rate occurred when the nucleophile (NaOH) concentration was dropped by 75% at a constant ionic strength.

Also, the rate of both the G-O and O-A bond cleavage was reduced when the concentration of the nucleophile was reduced (Table 1). This indicates that mechanisms other than an S_N1 are involved in the degradation reaction at both bond points.

S_Ni Mechanisms

An increase in NaOH concentration favors an S_Ni type of mechanism since more of the prerequisite C2 oxyanion would be produced. The $S_NicB(2)$ neighboring-group mechanism, shown in Figure 1, had been found to be a viable one for most of the previous studies of glycosidic bond cleavage. As with the S_N1 mechanism, the 1,5-anhydro-D-glucitol (**6**) would be formed with no ^{18}O incorporation if the reaction was done in ^{18}O-water.

However, a standard chair-to-chair ring flip (as shown in Figure 1) is not possible with the 4,6-O-benzylidene cellobiitol, since the ring fusion between the benzylidene and the glucose cannot invert to a diaxial geometry. The $S_NicB(2)$ might be possible with **4** by way of a boat conformation. One would anticipate that a reaction requiring a strained boat conformation would be much slower in rate (ca. 10 times slower) than one involving a chair conformation. Yet, the rate of G-O bond cleavage for the benzylidene disaccharide was still ~75% of that of the unsubstituted disaccharide (Table 1).

The $S_NicB(2)$-ro mechanism does not require a ring flip to a less stable conformation in either the benzylidene substituted or unsubstituted 1,5-anhydrocellobiitol. Instead, the first step of this mechanism consists of formation of the conjugate base by ionization of the C2 hydroxyl group (Figure 1). The conjugate base is set up for a backside attack at C1, with subsequent opening of the pyranoside ring. This 1,2-

epoxide intermediate is opened up by hydroxide ion in the reaction medium. The resulting hemiacetal converts to the aldehyde, kicking out the alkoxyl group (the aglycon) to give 1,5-anhydro-D-glucitol (**6**). The 1,5-anhydro-D-glucitol would have no ^{18}O incorporation in this case. The glycosyl side of the model compound gives the base-labile 4,6-O-benzylidene-D-glucose, the acyclic form of **9**.

S_N2 Mechanism

An S_N2 mechanism, which involves backside attack by a hydroxide ion with simultaneous cleavage of the G-O bond,[15] would also give 1,5-anhydro-D-glucitol with no ^{18}O incorporation from ^{18}O-water. The S_N2 mechanism has been ruled out for 1,5-anhydrocellobiitol based on the finding that the degradation rate did not increase in the presence of iodide or sulfide ion, which are stronger nucleophiles than hydroxide ion.[7] Likewise, we do not expect the S_N2 mechanism to be involved for the 4,6-O-benzylidene of 1,5-anhydrocellobiitol, since it is structurally similar to the 1,5-anhydrocellobiitol.

Conclusions on G-O Bond Cleavage

Clearly, the results of this study are best explained by an $S_N$icB(2)-ro mechanism for the G-O bond cleavage. The possible existence of a parallel, less dominant S_N1 mechanism cannot be ruled out.

Mechanisms of Oxygen-Aglycon (O-A) Bond Cleavage

S_N1 Mechanism

An S_N1 mechanism for O-A bond cleavage, shown in Figure 3, would produce an oxyanion on the glycosyl side and a carbocation on the aglycon side at the C4. The oxyanion of 4,6-O-benzylidene-D-glucopyranose would degrade to acidic products under the reaction conditions. On the aglycon side, the carbocation may simply be attacked by hydroxide ion to produce both 1,5-anhydro-D-glucitol and 1,5-anhydro-D-galactitol. Since the hydroxyl group at the C4 position would be provided from the reaction medium, these compounds

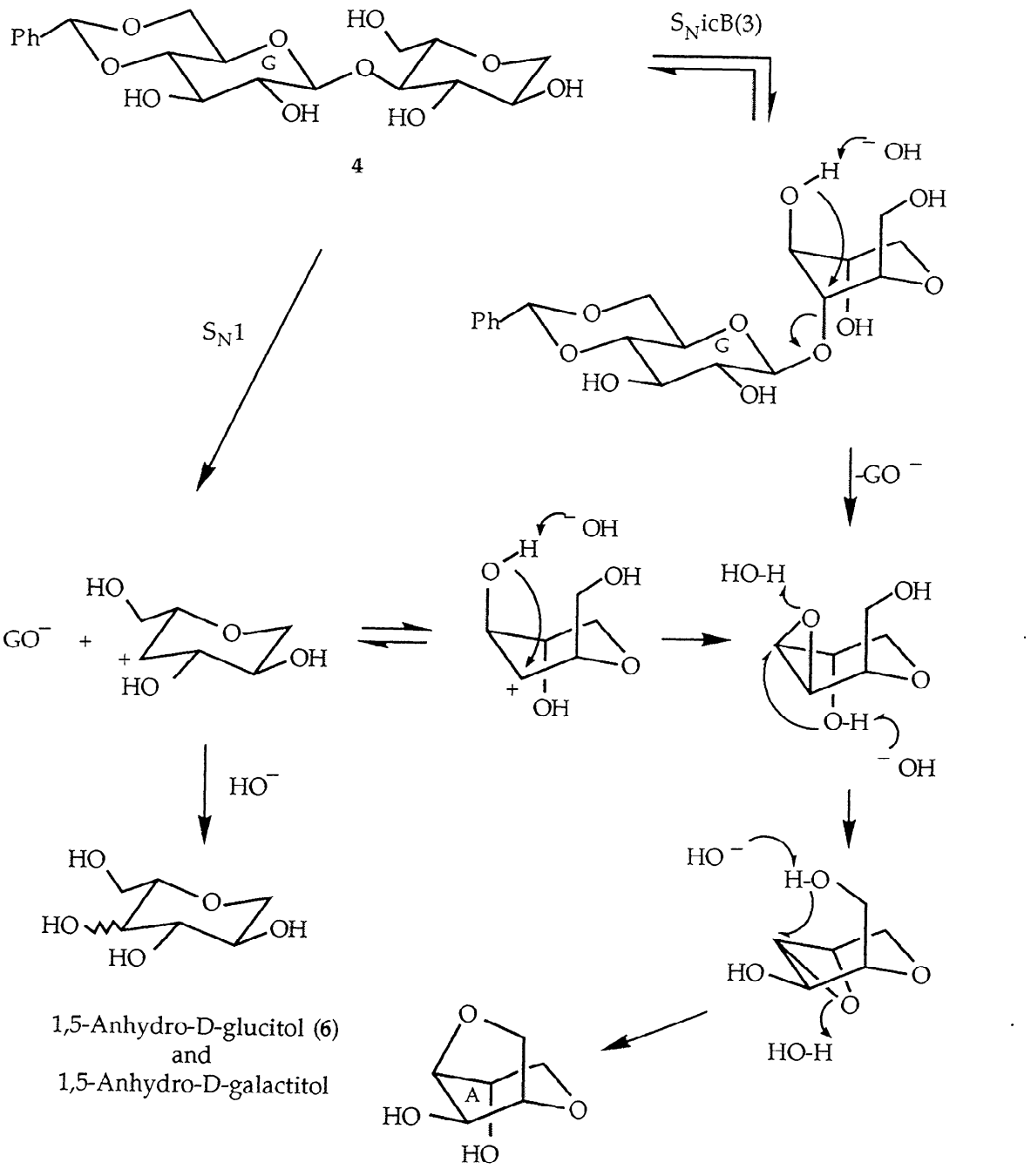

Figure 3. Mechanisms for Oxygen-Aglycon (O-A) Bond Cleavage for 4.

would be expected to be enriched in ^{18}O if the reaction was conducted in ^{18}O-enriched water. Analysis of the degradation products showed no detectable amount of 1,5-anhydro-D-galactitol (by comparison to a known standard[16]) or ^{18}O-enriched 1,5-anhydro-D-glucitol.

Yet, these two negative observations do not rule out an S_N1 mechanism, since the carbocation could instead undergo intramolecular nucleophilic attack by the C3 hydroxyl group (Figure 3). The resulting 3,4-epoxide could then migrate to form the 2,3-epoxide, followed by internal attack by the C6 hydroxyl at C3 to form 1,5:3,6-dianhydro-D-galactitol (**7**), a product which was formed in high yield. This product would not be expected to incorporate any ^{18}O in an enriched medium. Mass spectrometry confirmed this; no ^{18}O-enrichment was found in product **7**. However, the slower rate of formation of **7** when reduced levels of NaOH were employed does <u>not</u> support an S_N1 mechanism.

S_Ni Mechanism

The rate of a neighboring-group type reaction would be reduced when the NaOH concentration is reduced. The $S_N$icB(3) mechanism for O-A bond cleavage is illustrated in Figure 3. In the first step, the pyranose ring of the aglycon must flip to an all-axial conformation in order to achieve the required trans-diaxial conformation between the O-A bond and the C3 conjugate base. This allows backside attack by the C3 conjugate base, displacing the glycosyl side of the molecule. The glycosyl side becomes the base-labile 4,6-O-benzylidene-D-glucopyranose (**9**). The aglycon side, which now consists of the 3,4-epoxide, undergoes similar rearrangements as shown for an S_N1 mechanism to give 1,5:3,6-dianhydro-D-galactitol (**7**), a product observed in 32% yield.

S_N2 Mechanism

An S_N2 O-A bond cleavage would produce 1,5-anhydro-D-galactitol and 4,6-O-benzylidene-D-glucopyranose (**9**) anion. This mechanism can be ruled out since no (base-stable) 1,5-anhydro-D-galactitol was detected in the degradation products. This product was also not observed in alkaline degradations of 1,5-anhydrocellobiitol.[7]

Conclusions on O-A Bond Cleavage

The neighboring-group $S_N$icB(3) mechanism was concluded to be the most feasible. An S_N1 mechanism with subsequent epoxide formation and rearrangement cannot be ruled out. Both mechanisms would explain the formation of 1,5:3,6-dianhydro-D-galactitol.

Rate Differences of Substituted and Unsubstituted 1,5-Anhydrocellobiitol

The relative rates of degradation of 4,6-O-benzylidene 1,5-anhydrocellobiitol (**4**) and 1,5-anhydrocellobiitol (**8**) under identical conditions were quite different; the benzylidene degraded ~35% faster. The rate difference can be attributed to a much faster rate of O-A bond cleavage for the benzylidene. As indicated by the data in Table 1, the rate of O-A bond cleavage was 350% faster for the benzylidene, while the rate of G-O bond cleavage was 30% slower for the benzylidene.

The rate increase for O-A cleavage for the benzylidene could reflect that the benzylidene substituted glucoside is a better leaving group than an unsubstituted glucoside. In a highly alkaline medium, several of the carbohydrate hydroxyl groups will be ionized or pseudo-ionized. The benzylidene glucoside has two hydroxyl groups (at C3 and C4), while the unsubstituted glucoside has four hydroxyl groups (at C2, C3, C4, and C6); thus, the latter will likely be charged to a greater extent in NaOH solution. A more highly charged glucoside represents a poorer leaving group in a rate determining S_N2, S_N1, or $S_N$icB(3) mechanism.

The fact that G-O bond cleavage is 30% faster in the unsubstituted 1,5-anhydrocellobiitol may indicate that both $S_N$icB(2) and $S_N$icB(2)-ro mechanisms are operating in this case, while only the ring opening mechanism operates in the benzylidene case. The data suggest that the ring opening mechanism could be the dominant G-O bond cleavage mechanism for both 1,5-anhydrocellobiitol models. Since pyranose ring flips should be difficult for carbohydrate polymers, random chain cleavage reactions of cellulose are probably also occurring largely by an $S_N$icB(2)-ro mechanism.

EXPERIMENTAL

Analytical Methods

Melting points were measured on an Electrothermal® melting point apparatus. TLC was done on plates coated with 60Å-silica gel (250 μm layer thickness). The plates were developed using sulfuric acid:methanol (1:4, v:v) followed by charring to detect the components on the slide. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory (Woodside, New York).

The ^{1}H and ^{13}C-NMR spectra were recorded using a Bruker AC 250 MHz or a Varian Gemini 300 MHz spectrometer; tetramethylsilane was added as an internal standard. Gas chromatography (GC) was done on a Hewlett Packard 5890 Series II GC equipped with a flame-ionization detector (FID) and fused silica capillary columns from J & W Scientific (30 m long, 0.25 micron film thickness, type DB1-30N). Hydrogen was used as the carrier gas. Split injections were done using a 50:1 split ratio. The analysis conditions were: injector - 275°C; detector - 250°C; column -180°C for 1 min, ramp 20°C/min to 300°C, hold for 5 min at 300°C.

Response factors relative to inositol (the internal standard) were determined for each compound to obtain quantitative information from the GC analysis. Mass spectral analyses were performed on a Vacuum Generators Micromass Model 7070E, equipped with a VG-11-250J Data System Computer. Samples were introduced after separation via GC, using a DB5 fused silica capillary column and FID.

Reagents/Compounds

Oxygen-free water was prepared by boiling distilled, deionized water, and then purging it with nitrogen while cooling to room temperature in ice. Ammonia/methanol mixtures were prepared by sparging freshly distilled methanol with ammonia gas until saturated (about 15 minutes). The silylating reagent was Tri-Sil®, obtained from Pierce

in 1-mL ampoules. ^{18}O-Enriched water (98 atom %) was obtained in 1.0-g ampoules from Icon Services.

4,6-O-Benzylidene-1,5-anhydro-D-glucitol (5). The 1,5-anhydro-D-glucitol (0.505 g, 0.00308 mol) was mixed with N,N-dimethylformamide (17 mL), α,α-dimethoxytoluene (0.68 g, 0.00447 mol), and a catalytic amount of *p*-toluenesulfonic acid (ca. 0.04 g). The reaction flask was attached to a rotary evaporator, and evacuated using a water-aspirator vacuum (ca. 70 mm Hg). The flask and its contents were heated to 60°C, and the vacuum was adjusted so that the DMF refluxed gently.

After 70 minutes, the mixture was allowed to cool to room temperature. Following neutralization with triethylamine or pyridine (5 mL), the mixture was concentrated *in vacuo* (50°C, 2 mm Hg). The resulting crude solid was purified on a silica gel (Merck grade 60, 230-400 mesh) column with ethyl acetate-dichloromethane (90:10, v:v), to give 0.62 g (80% yield) of **5**: mp 164.0-166.5°C. The sample was further purified by slurrying a solution of the solid (in chloroform/methanol) with activated carbon, and filtering. The filtrate was concentrated *in vacuo* to give an off-white solid: mp 163.5-165.0°C; ^1H-NMR ($CDCl_3$/MeOH) δ 3.3-4.4 (m, 14 H, -C<u>H</u>O<u>H</u>), 5.6 (s, 1 H, $C_6H_5C\underline{H}$-), 7.4-7.5 (m, 5H, -$C_6\underline{H}_5$); ^{13}C-NMR ($CDCl_3$/MeOH) ppm 69.0, 70.1, 70.2, 71.2, 75.3, 81.5, 102.0 ($C_6H_5\underline{C}H$-), 126.5, 128.4, 129.3, 137.4 (-$\underline{C}_6H_5$); EI-MS *m/e* 252 (M^+), 179, 160, 129, 105, 91, 73, 57, 43.

Anal. Calcd. for $C_{13}H_{16}O_5$: C, 61.90; H, 6.35%. Found: C, 61.90; H, 6.57.

2,3,6-Tri-O-acetyl-1,5-anhydro-4-O-(2,3-di-O-acetyl-4,6-O-benzylidene-β-D-glucopyranosyl)-D-glucitol. 1,5-Anhydro-4-O-β-D-glucopyranosyl-D-glucitol (1,5-anhydrocellobiitol) (**8**)[17] (0.51 g, 0.00157 mol) was mixed with freshly distilled N,N-dimethylformamide (17 mL), α,α-dimethoxytoluene (0.34 g, 0.00226 mol), and a catalytic amount of *p*-toluenesulfonic acid (0.04 g). The reaction flask was attached to a rotary evaporator evacuated to ~70 mm Hg, and heated to 60°C; the vacuum was adjusted so that the DMF refluxed gently.

After 5 hours, the reaction mixture was stoppered and allowed to cool to room temperature. Pyridine (5 mL) was added, and the mixture

was concentrated *in vacuo* (50°C, 5 mm Hg) to give a beige solid, crude **4**. The crude solid **4** was acetylated using pyridine (6 mL) and acetic anhydride (2 mL). The mixture was stirred at room temperature for four days, then poured into ice-water (150 mL); a cloudy white precipitate formed. The ice/water was extracted with chloroform (4 x 100 mL), and the chloroform extracts were washed with water (3 x 100 mL), dried over anhydrous potassium carbonate, filtered, and concentrated *in vacuo* to a white solid (1.0 g): mp 217-222°C. A portion of the crude solid was recrystallized from isopropyl alcohol/pyridine (50:1, v/v), which gave a 45% overall yield of the acetylated compound: mp 238.0-240.5°C (Lit.[18] mp 222-225°C); ^{1}H-NMR (d_6-DMSO) δ 1.9-2.1 (m, 15 H, -COC$\underline{H}_3$), 5.6 (s, 1 H, $C_6H_5C\underline{H}$-), 7.4 (m, 5 H, -$C_6\underline{H}_5$). This spectrum is identical to that done for a previously prepared sample.[18]

1,5-Anhydro-4-O-(4,6-O-benzylidene-β-D-glucopyranosyl)-D-glucitol (4). The acetylated compound above (0.25 g) was deacetylated by adding anhydrous methanol which had been saturated with ammonia gas (30 mL). After stirring for 16 hours at room temperature, the mixture was concentrated *in vacuo* to yield a white solid. The product, **4**, was recovered in essentially quantitative yield: mp 118-130°C (Lit.[18] mp 95-115°C); ^{1}H-NMR (d_6-DMSO) δ 5.59 (s, 1 H, $C_6H_5C\underline{H}$), 7.4-7.5 (m, 5 H, -$C_6\underline{H}_5$); ^{13}C-NMR (d_6-DMSO) ppm 61.0, 66.5, 68.2, 69.9, 70.4, 73.4, 75.0, 76.8, 80.1, 80.2, 80.9, 101.2, 103.7, 126.9, 128.6, 129.4, 138.3. Both the ^{1}H and ^{13}C-NMR spectra were comparable to those done for a previously prepared sample of compound **4**.[18]

4,6-O-Benzylidene-D-glucopyranose (9). This compound was prepared using a literature procedure:[19] mp 185.5-187.0°C (Lit.[19] mp 186-187°C).

Thermal Degradation Studies

Degradation studies of the carbohydrate samples were conducted in 4-mL stainless steel pressure vessels ("bombs"). The carbohydrate samples (0.03-0.04 mmoles per sample) were accurately weighed into tared reaction bombs. The appropriate amount of standard sodium

hydroxide solution (2.78 N, or 2.5 N NaOH at 170°C) was added to each sample; typically, a 250-fold excess of NaOH to the carbohydrate was used. The reaction bombs were then sealed, weighed for total weight, and lowered into a preheated fluidized sand bath (at 170°C). The bombs were held in place with a plate, which was attached to a shaker arm so that the samples could be mixed during the studies.

Some experiments were conducted in an O_2-free atmosphere; the samples were prepared with O_2-free water under nitrogen in a glove bag. Degradation rates and product distributions were the same as those which took no precautions to eliminate oxygen.

In the study where a lower hydroxide concentration at the same ionic strength was evaluated, a solution of 2.78 N sodium chloride was added in the appropriate amount to the reaction bomb after the carbohydrate sample and the standard 2.78 N NaOH solution had been added. For the ^{18}O labeling experiment, a 1.00-mL aliquot of 5.56 N NaOH solution was added to the bomb in a nitrogen atmosphere. This was immediately followed by a 1.0-g ampoule of ^{18}O-enriched water (98 atom % purity); the actual amount added was determined gravimetrically (2.83 N, or 2.54 N at 170°C). At the same time, "control" samples were prepared containing 2.8 N NaOH which was not enriched in ^{18}O.

Samples were pulled at various times (2-98 hours) for kinetic analysis. Once pulled from the sand bath, the reaction bomb samples were allowed to cool to room temperature, and reweighed to verify that the bombs had not leaked during the heating. The bombs were opened, and an internal standard (aq. inositol solution, ca. 0.02 mmol) was added directly to the bombs. The resulting mixture was neutralized using a 5% excess of aqueous ammonium chloride (20% w/w). For GC analysis, an aliquot of the sample stock solution was concentrated in vacuo (50°C, 5 mm Hg). A 1-mL ampoule of Tri-Sil® reagent was added to the sample flask, and the mixture was silylated using a method based on that by Sweeley et al.[20] The silylated samples were centrifuged, and the supernatant analyzed by GC. In the case of the ^{18}O experiment, MS was used to determine if ^{18}O had been incorporated into any of the degradation products.

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